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Quantitation of chlorophenoxy acid herbicides by high-performance liquid chromatography with coulometric detection

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Abstract

Chlorophenoxy acids are toxic compounds used as selective herbicides in agriculture. To meet the need to determine trace levels of such pesticides in ground and drinking water, a highly selective and sensitive method using HPLC in combination with electrochemical detection has been developed. The four phenoxy acids 4-chloro-2-methylphenoxyacetic acid (MCPA), 4-(4-chloro-2-methylophenoxy)butyric acid (MCPB), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T) were investigated coulometrically in the oxidative mode. To determine the optimum working potential, hydrodynamic voltammograms were recorded at potentials between +0.1 and +1.0 V. As electrochemical activity is present only at higher potentials, the influence of photolysis was investigated with a view toward improving selectivity and sensitivity. After photoconversion by UV irradiation, detection ensued at potentials 0.3 V lower than without photolysis. For efficient preconcentration prior to HPLC-electrochemical detection, a solid-phase extraction system with C_{18} cartridges was employed, resulting in high recovery and good reproducibility. The method developed meets the requirements with regard to the limits for pesticides in ground and drinking water and may be useful for the analysis of environmental herbicide residues.

Keywords: Water analysis; Environmental analysis; Chlorophenoxy acids; Pesticides

1. Introduction

Several hundred pesticides of different chemical structure are used world-wide in agriculture. In Germany alone, a total of 32.5 million kilograms of herbicides are used in agriculture every year [1]. Apart from herbicides, fungicides, insecticides and bactericides are applied as well, but their proportion to the total pesticide sum has fallen under 45%. Thus herbicides have to be considered to be of primary importance.

Owing to their high toxicity, it was necessary to set limits for all the pesticides in drinking water, which are 0.1 $\mu g\,l^{-1}$ for individual compounds and 0.5 $\mu g\,l^{-1}$ for the sum of all pesticides [2].

According to literature, a pesticide is able to contaminate ground water if its water solubility is higher than 30 $mg\,l^{-1}$, its adsorptivity is less than 300–500 $ml\,g^{-1}$, its soil half-life is longer than 2–3 weeks, its hydrolysis half-life is longer than about six months and its photolysis half-life is longer than three days [3]. Other relevant parameters are described in a review by Barcelo [4].

Chlorophenoxy acid herbicides in particular are

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widely used for the postemergence treatment of grain, corn, rice and sugar cane. Because the free acid and the salt form are highly soluble in water, they are not adsorbed in the upper layers of the soil, but may leach down into ground water and later contaminate drinking water. The ester form of those acids can then accumulate in the fat of aquatic and terrestrial organisms [5]. Thus there is the need for a highly selective and sensitive method for the determination of chlorophenoxy acids in ground and drinking water as well as in aquatic organisms in conjunction with a suitable sample pretreatment.

In literature, various chromatographic methods are described for this purpose. Beside capillary electrophoresis [6], gas chromatography in combination with mass spectroscopy [7] or selective detectors like electron capture [8–12] are used for environmental herbicide analysis.

For the determination of chlorophenoxy acid herbicides various methods using HPLC in combination with UV detection and off-line sample preparation have already been described [13–17]. In contrast to gas chromatography, liquid chromatographic methods have the advantage of being suitable for thermally labile and polar herbicides as well, as these substances require derivatization prior to gas chromatographic investigations.

This paper deals with a new method using HPLC in conjunction with electrochemical detection (ED) for the analysis of 4-chloro-2-methylphenoxy acetic acid (MCPA), 4-(4-chloro-2-methylphenoxy)butyric acid (MCPB), 2,4-D and 2,4,5-T, four of the most important phenoxy acids, in ground and drinking water. To obtain efficient preconcentration with good reproducibility and accuracy a C_{18} solid-phase extraction system was applied.

2. Experimental

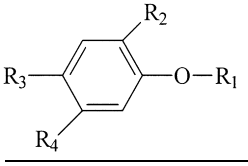
2.1. Material

The four investigated herbicides MCPA, MCPB, 2,4-D and 2,4,5-T were obtained from Labor Dr. Ehrenstorfer (Augsburg, Germany). Their chemical structures are shown in Table 1. Methanol and acetonitrile of HPLC grade were purchased from Merck (Darmstadt, Germany). Water for the mobile phase was deionized, distilled twice in a quartz still and further purified with a cartridge purification system (Nanopure, Barnstead). Solid-phase extraction was carried out with Bond Elut C_{18} cartridges, 1000 mg, ICT (Vienna, Austria), Bakerbond spe C_{18} cartridges, 1000 mg, J.T. Baker (Germany) and LiChrolut EN cartridges, 200 mg, Merck. All other solvents were of analytical grade.

2.2. RP-HPLC

The HPLC system consisted of a Merck–Hitachi L-6200A intelligent pump, which was connected via a Merck–Hitachi D-6000 Interface to a personal computer. The system used the HM software from Merck. UV detection was performed with a Merck–Hitachi L-4250 UV–Vis detector at 254 nm. Electrochemical detection was carried out by a 5100A ESA Coulochem (Vienna, Austria) with a dual electrode analytical cell and a single electrode guard cell. The

Table 1
Structure of investigated chlorophenoxy acid herbicides



Herbicide	IUPAC-Nomenclature	R_1	R_2	R_3	R_4
MCPA	4-Chloro-2-methylphenoxyacetic acid	$-CH_2-COOH$	$-CH_3$	-Cl	-H
MCPB	4-(4-Chloro-2-methylphenoxy)butyric acid	$-(CH_2)_3-COOH$	$-CH_3$	-Cl	-H
2,4-D	2,4-Dichlorophenoxyacetic acid	$-CH_2-COOH$	-Cl	-Cl	-H
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid	$-CH_2-COOH$	-Cl	-Cl	-Cl

analytical cell was installed after the analytical column, a Merck LiChroCart 125×4 mm HPLC cartridge packed with LiChrospher 100, RP18, 5 μm. The guard cell was installed between pump and injection valve in order to electrooxidize impurities in the mobile phase. To further protect both the analytical column and the analytical cell a precolumn was used, which was packed in our laboratory with Perisorb RP18, 30–40 μm, Merck. UV irradiation was provided by a Beam Boost Reaction Unit, ICT, with a Sylvania GTE 8W low pressure UV lamp, emission maximum 254 nm, and a PTFE irradiation coil, length 3 and 7 m, I.D. 0.3 mm and O.D. 1.3 mm.

The mobile phase consisted of methanol–water containing 2 g lithiumperchlorate per litre as leading salt and 2 ml glacial acetic acid for a pH adjustment to 3.9. The prepared mobile phases were filtered through a 0.2 μm membrane filter, Sartorius, and then degassed with helium 5.0. The HPLC system was conditioned by passing the mobile phase through it for 2 h at a flow-rate of 0.5 ml min^{-1}.

2.3. Stock solutions and calibration

Stock solutions were made by dissolving 4 mg of the respective herbicide in 99 ml methanol and 1 ml glacial acetic acid. The solutions were further diluted 1:10 with methanol and injected into the HPLC system.

Calibration for use in environmental herbicide analysis was done by spiking water with the stock solutions at concentrations between 500 ng and 1 ng per ml.

2.4. Hydrodynamic voltammograms

In order to determine the optimum working potential of the analysed herbicides, hydrodynamic voltammograms were recorded by injecting samples with a set concentration of 1 μg ml^{-1} at different working electrode potentials. Investigations were started at the highest potential of +1.0 V and then slowly decreased in 0.1 V steps. Hydrodynamic voltammograms were obtained by plotting the measured peaks against the applied potentials.

2.5. Sample preparation

2.5.1. Using Bond Elut and Bakerbond C_{18} cartridges

The extraction cartridges were preconditioned with 5×1 ml methanol and 5×1 ml bidistilled water. Then 500 ml of the prepared sample adjusted to a pH of 2.0 (water spiked with stock solution containing between 500–10 ng ml^{-1} and, respectively ground water from the south of Styria) were transferred onto the cartridges. Vacuum was applied and the eluates were discarded. After washing each cartridge with 5 ml bidistilled water, the columns were dried under a vacuum of 10 mmHg for 20 min (1 mmHg=133.322 Pa). The phenoxy acids were washed off with 3×1 ml acetonitrile or methanol, respectively and the eluates concentrated by evaporation to dryness. The residues were redissolved in 0.1 ml of acetonitrile–water (15:85, v/v) and 0.020 ml injected into the HPLC system.

2.5.2. Using LiChrolut EN

After preconditioning the SPE cartridges with 3 ml methanol, 3 ml bidistilled water and 3 ml bidistilled water adjusted to pH 3.0, 500 ml sample pH 3.0 were transferred to them. Each cartridge was washed with 1 ml bidistilled water to remove water-soluble impurities and then dried under a vacuum of 10 mmHg for 60 min.

Finally the chlorophenoxy acids were eluted with 4×1 ml methanol or acetonitrile and concentrated to dryness by a stream of nitrogen. The residues were dissolved in 0.1 ml of the mobile phase, methanol–water (3:2, v/v) and 0.02 ml of these solutions were injected into the HPLC system.

3. Results and discussion

3.1. Chromatography

First, to determine the optimum chromatographic analysis and separation conditions of the four chlorophenoxy acids an UV detector was used instead of an electrochemical detector, because of its easier handling and lower running costs. To evaluate the mobile phase different ratios of methanol–water were tested with respect to optimal peak sharpness and time of

Table 2
HPLC conditions for the analysis of the studied chlorophenoxy acids. Column, LiChrospher 100, RP 18, 125×4 mm, 5 μm; precolumn, Perisorb, RP 18, 30–40 μm; electrochemical detection, potential 0.9 V, with photolysis, irradiation at 254 nm, 7 m PTFE irradiation coil, I.D. 0.3 mm, O.D. 1.3 mm; flow-rate, 0.5 ml min^{-1}; concentration, 20 ng of each herbicide per injection

Mobile phase	Retention time (min)				Peak symmetry			
	MCPA	MCPB	2,4-D	2,4,5-T	MCPA	MCPB	2,4-D	2,4,5-T
Methanol–water (255:245, v/v), $LiClO_4$ 2 g l^{-1}, acetic acid 2 ml l^{-1}	8.53	24.12	7.32	13.10	1.64	1.59	1.50	1.81
Methanol–water (275:225, v/v), $LiClO_4$ 2 g l^{-1}, acetic acid 2 ml l^{-1}	7.55	22.41	6.55	12.30	1.61	1.53	1.46	1.74
Methanol–water (300:200, v/v), $LiClO_4$ 2 g l^{-1}, acetic acid 2 ml l^{-1}	6.27	19.02	5.00	11.30	1.12	1.46	1.09	1.33
Methanol–water (320:180, v/v), $LiClO_4$ 2 g l^{-1}, acetic acid 2 ml l^{-1}	3.55	17.59	3.00	8.70	1.14	1.40	1.11	1.29

retention. Considering the fact that, at flow-rates higher than 0.7 ml min^{-1}, electrooxidation at the working electrode does not occur quantitatively, the flow has to be lower. Furthermore, the guard cell and the analytical cell as well as the graphite filters in the electrochemical detection system increased the pressure resistance of the HPLC, which also represented a limiting factor for the flow-rate. Therefore, a methanol–water mixture with a ratio of 3:2 (v/v) at a flow-rate of 0.5 ml min^{-1} proved to be the most suitable mobile phase with regard to peak form, retention time and chromatographic separation of the four investigated herbicides.

The HPLC conditions including all the examined mobile phases and the corresponding times of retention as well as peak symmetry are summarized in Table 2. As can be seen, the greatest differences in the retention times between MCPA and 2,4-D can be obtained using the 3:2 ratio, without significantly extending of the retention time of MCPB.

3.2. Electrochemical activity

In analogy to different drugs [18–20] and as described in literature for chloroaromatic compounds [21–23], also chlorophenoxy acids have to possess electrochemical activity due to their structure. Fig. 1 shows the scheme of reaction on the example of MCPB. As generally known, the chlorine substituent of the aromatic ring is exchanged by a hydroxyl function in aqueous or methanolic solution because of photooxidation and subsequently the phenolic hydroxyl is further oxidated, so causing an electrochemical signal.

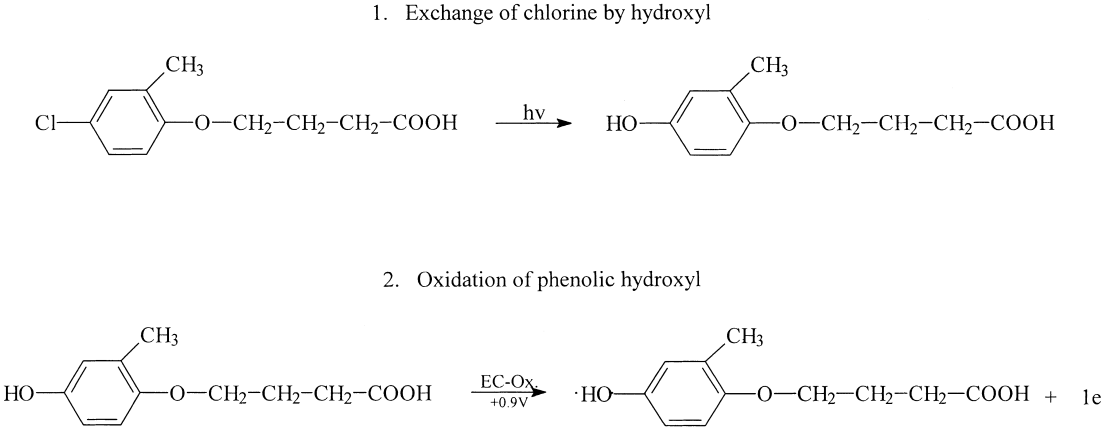


Fig. 1. Scheme of reaction on the example of MCPB.

Comparing the intensities of the signals for MCPA, MCPB, 2,4-D and 2,4,5-T, MCPA shows the highest and 2,4,5-T the lowest peak signal, whereas MCPB is not electrochemically active at any potential without irradiation. This behaviour may be explained by the butyric acid structure of the sidechain, which is the only difference to the acetic acid sidechain of MCPA. The electrochemical response for 2,4-D is about 75% smaller than the signal for MCPA, but double the intensity for 2,4,5-T. The different electrochemical behaviour of the four herbicides may be explained by the varying number of chlorine atoms in the aromatic moiety and the different aliphatic sidechain. MCPA has one chlorine atom in position 4 of the benzene ring, which can be transformed easily and without photolysis into an electrochemically active phenolic derivative.

3.3. Influence of UV irradiation

UV irradiation may increase selectivity as well as sensitivity of the electrochemical reaction, as the photolytic process makes the transformation of the chlorine substituent easier because the activation energy is reduced. Thus, after photoconversion by UV irradiation, electrochemical detection ensues on average at potentials 0.3 V lower than without photolysis. Besides, due to the lower detection potential, background current is lower as well and the baseline is more stable, resulting in better detection limits (Fig. 2).

Table 3
Electrochemical activity of MCPA, MCPB, 2,4-D and 2,4,5-T with and without UV irradiation

Herbicide	Signal intensity (V)	
	Without photolysis	With photolysis
2,4-D	0.101	0.065
2,4,5-T	0.050	0.099
MCPA	0.398	0.404
MCPB	0	0.192

However, the electrochemical intensity of MCPA is not influenced significantly by UV irradiation, only the detection potential is lowered about 0.1 V. In the case of 2,4-D and 2,4,5-T, it could be observed that the electrochemical response with irradiation is opposite to the peak signals without photolysis. This means that, without irradiation, the intensity of the 2,4,5-T peak is about 50% lower than the signal for 2,4-D, but with irradiation the intensity of 2,4,5-T is nearly doubled, whereas the electrochemical response of 2,4-D is reduced about 30%. MCPB, which showed no electrochemical signal without photolysis, becomes electrochemically active with a signal intensity half the intensity of MCPA. Table 3 compares the peak intensities of MCPA,

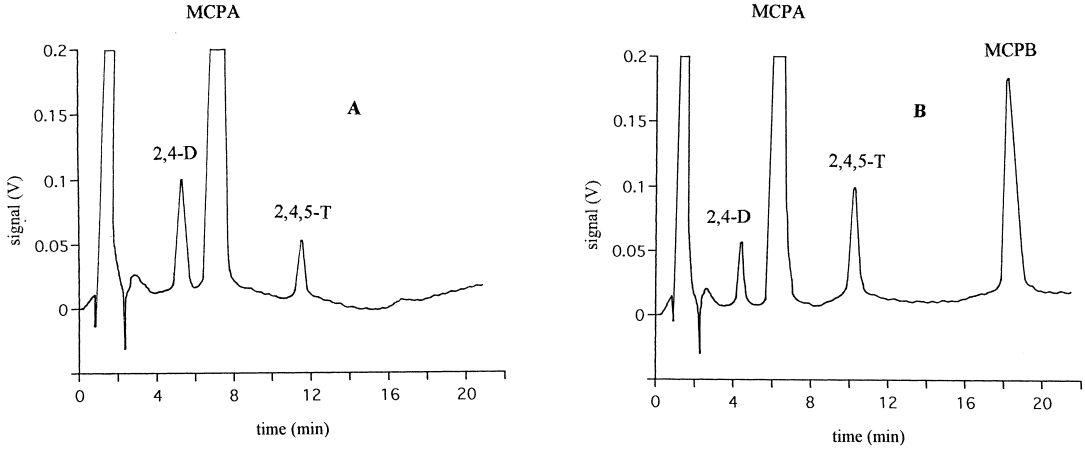


Fig. 2. Chromatogram of the phenoxy acids with and without UV irradiation. Column, LiChrospher 100, RP 18, 125×4 mm, 5 μm; mobile phase, methanol–water (3:2, v/v), lithium perchlorate 2 g l^{-1}, acetic acid 2 ml l^{-1}; potential, 0.9 V; flow-rate, 0.5 ml min^{-1}; concentration, 10 ng of each herbicide per injection. (A) Without photolysis. (B) With photolysis.

MCPB, 2,4-D and 2,4,5-T with and without photolysis at a working potential of +0.9 V.

3.4. Optimum working potential

Hydrodynamic voltammograms were recorded to determine the optimum working potential for the electrochemical investigation of the chlorophenoxy acid herbicides. The voltammograms were obtained by injecting the herbicide solution with a constant concentration of 1 μg ml^{-1} into the HPLC system while the potential of the working electrode was

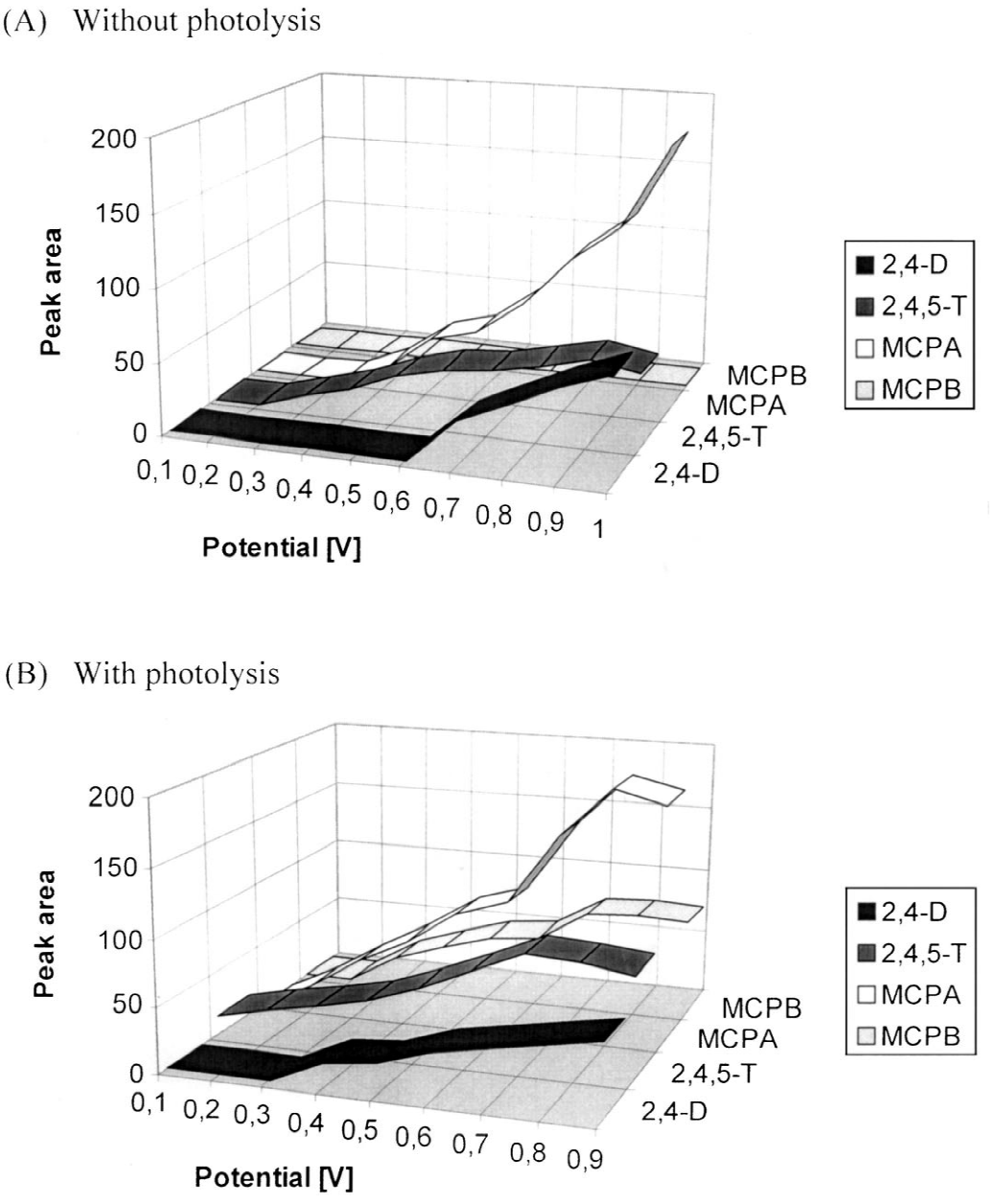


Fig. 3. Hydrodynamic voltammograms of the herbicides with and without photolysis. Column, LiChrospher 100, RP 18, 125×4 mm, 5 μm; mobile phase, methanol–water (3:2, v/v), lithiumperchlorate 2 g l^{-1}, acetic acid 2 ml l^{-1}; potential, 0.9 V; flow-rate, 0.5 ml min^{-1}; concentration, 20 ng of each herbicide per injection. (A) Without photolysis. (B) With photolysis.

scaled down from +1.0 V to +0.1 V. Background current was allowed to stabilize for 10 min before each injection.

To evaluate the optimum electrochemical conditions with regard to sensitivity and selectivity as well as background current and baseline drift, hydrodynamic measurements were carried out with and without photolysis.

As illustrated in Fig. 3, 2,4-D, 2,4,5-T as well as MCPA show maximum electrochemical response at potentials about +0.9 and +1.0 V without UV irradiation. With the exception of 2,4-D the substances become electrochemically detectable at +0.3 V. MCPB does not possess electrochemical activity without photolysis at any working electrode potential, whereas, in combination with UV irradiation, MCPB exhibits electrochemical properties similar to MCPA due to their related chemical structures. Both reach their maximum response at +0.85 V and are detectable in a range from +0.2 V and +0.3 V, respectively, to +0.9 V. In contrast, 2,4-D shows electrochemical activity from +0.45 V to +0.9 V and 2,4,5-T from +0.05 to +0.9 V; the maximum is achieved at +0.75 V for the latter. However, at potentials higher than +0.9 V with and +1.0 V without photolysis background current increases because of electrooxidation of possible impurities in the mobile phase resulting in a decrease in the signal-to-noise ratio and lowered detection signals. Thus a working potential of +1.0 V was chosen for the determination of the chlorophenoxy acids without photolysis and +0.9 V for their analysis with UV irradiation.

3.5. Solid-phase extraction

Two different C_{18} cartridges and a specially designed extraction column were employed for the pretreatment of the water samples. The exact extraction procedure is described in the experimental section.

An important parameter for obtaining high recovery rates was the adjustment of the test solutions to a pH value of 2 or 3 for use with LiChrolut EN cartridges, as the enrichment of the chlorophenoxy acids requires acidic conditions. Furthermore, a drying step for the sorbent after washing out water soluble impurities contained in the samples was necessary with regard to recovery and reproducibility. Otherwise, if the sorbent is moist, extraction may occur inhomogeneously and more impurities may be eluted, disturbing analysis.

Freeze-drying or drawing air or nitrogen through the cartridges are possibilities for adequate drying. However, drying the sorbent by drawing air under vacuum through the cartridges for 20 min or 60 min in the case of LiChrolut EN extraction columns, and an additional storage of the dried cartridges in a freeze-dryer overnight proved to be the best method, resulting in recovery rates of about 95–100%. The elution of the enriched substances was carried out with methanol and acetonitrile, as only small amounts of those solvents are necessary for quantitative desorption. Table 4 compares the obtained recovery rates using methanol and acetonitrile for the elution of the herbicides from Bond Elut, Bakerbond and LiChrolut EN extraction cartridges. As can be seen, the employment of LiChrolut EN in combination with methanol as solvent for complete elution achieved the best results for recovery and reproducibility.

To evaluate this method, water spiked with herbicide stock solution containing 0.1 μg to 10 μg per l as well as well-water from near the city of Graz, Austria, were examined. In the case of the well-water an additional clean-up prior to SPE is required, if the water is cloudy or contains suspended material. This further pretreatment is done by filtering the water samples through a glass-fiber filter.

As Fig. 4 shows, no herbicide from the four chlorophenoxy acids was detectable in a well-water sample, either with or without UV irradiation.

3.6. Linearity and sensitivity

Calibration was carried out by repeatedly injecting water samples spiked with herbicide solutions in different concentration ranges into the HPLC–ECD system. Linear correlation was obtained in the range of 50–500 ng ml^{-1} for 2,4-D and 2,4,5-T, 10–50 ng ml^{-1} for MCPB and 2–10 ng ml^{-1} for MCPA. The coefficient of correlation for all measurements was higher than 0.998.

To determine the limits of detection, 20 μl aliquots with eight concentrations from 1 ng to 100 ng ml^{-1} corresponding to 1 pg to 100 pg ml^{-1} well-water after solid-phase extraction were analysed and

Table 4
Solid-phase extraction studies

Cartridge	Herbicide	Elution	Mean recovery (%) ±S.D. (n=5)	Notes
Bond Elut LRC C_{18}	MCPA	Acetonitrile	71.49±1.24	Adjustment of test
	MCPA	Methanol	95.34±0.12	solution to pH 2.0
	MCPB	Acetonitrile	76.21±4.13	
	MCPB	Methanol	89.97±1.07	
	2,4,-D	Acetonitrile	72.53±3.45	
	2,4-D	Methanol	96.00±2.23	
	2,4,5-T	Acetonitrile	79.04±1.17	
	2,4,5-T	Methanol	92.66±1.12	
Bakerbond spe C_{18}	MCPA	Acetonitrile	70.20±0.71	Adjustment of test
	MCPA	Methanol	92.67±1.14	solution to pH 2.0
	MCPB	Acetonitrile	71.13±4.21	
	MCPB	Methanol	94.61±2.07	
	2,4,-D	Acetonitrile	70.43±1.56	
	2,4-D	Methanol	93.12±5.19	
	2,4,5-T	Acetonitrile	81.14±0.68	
	2,4,5-T	Methanol	94.98±1.23	
LiChrolut EN	MCPA	Acetonitrile	89.23±4.17	Adjustment of test
	MCPA	Methanol	95.25±1.07	solution to pH 3.0
	MCPB	Acetonitrile	90.12±2.07	
	MCPB	Methanol	95.89±0.12	
	2,4,-D	Acetonitrile	83.33±2.08	
	2,4-D	Methanol	96.49±3.86	
	2,4,5-T	Acetonitrile	91.67±0.95	
	2,4,5-T	Methanol	105.55±2.06	

evaluated according to the peak signals obtained. Using such preconcentration up to 2 pg ml^{-1} (MCPA) can be determined.

3.7. Recovery and precision

Recovery and precision of the developed analysis

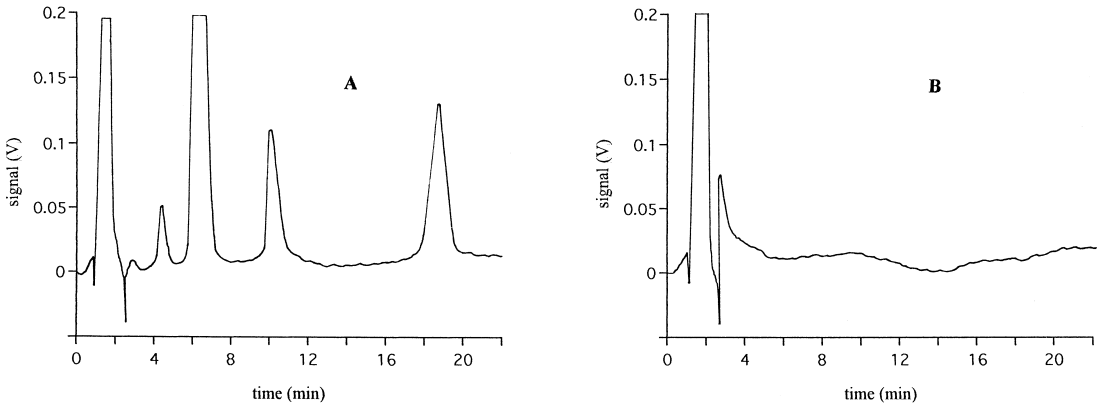


Fig. 4. Chromatogram after SPE with LiChrolut EN of well-water from near the city of Graz (Austria). Column, LiChrospher 100, RP 18, 125×4 mm, 5 μm; mobile phase, methanol–water (3:2, v/v), lithium perchlorate 2 g l^{-1}, acetic acid 2 ml l^{-1}; potential, 0.9 V; UV irradiation; flow-rate, 0.5 ml min^{-1}. (A) Spiked with phenoxy acids. (B) Without spiking.

Table 5
Recovery and precision results for the investigated chlorophenoxy acids in water

Herbicide	Concentration (mean±SD) (ng ml^{-1})		Recovery (%)	Precision (%)	Limit of detection (ng ml^{-1})
	Added	Found			
MCPA	250	244±12.93	96	5.3	0.002
	1000	1030±46.35	103	4.5	
MCPB	250	251±9.04	100	3.6	0.01
	1000	989±30.66	98	3.1	
2,4-D	250	239±9.80	92	4.1	0.03
	1000	1013±55.72	101	5.5	
2,4,5-T	250	260±10.14	104	3.9	0.1
	1000	1074±32.22	107	3.0	

method were determined by adding MCPA, MCPB, 2,4-D and 2,4,5-T in two different concentrations to blank water. The recovery for all four substances ranged between 92 and 107%, whereas the precision was ±5.5% at most. All statistical data including the limits of detection for the chlorophenoxy acid herbicides are listed in Table 5.

4. Conclusion

Due to the high toxicity of chlorophenoxy acid herbicides, a highly selective and sensitive method using HPLC in conjunction with ED was developed for their analysis in ground and drinking water. For the chromatographic separation of MCPA, MCPB, 2,4-D and 2,4,5-T, an RP18 column as stationary phase and a methanol–water (3:2, v/v) mixture containing glacial acetic acid and lithiumperchlorate as mobile phase proved to be appropriate. Measurements were carried out coulometrically in the oxidative mode. In order to determine the optimum working potential hydrodynamic voltammograms had to be recorded at potentials between +0.1 and +1.0 V. For the improvement of selectivity as well as sensitivity the influence of UV irradiation on the electrochemical behaviour of the herbicides was studied, resulting in lower detection potentials.

To determine concentrations in the picomol range an efficient preconcentration prior to HPLC–ED with high recovery and good reproducibility could be obtained with solid-phase extraction using three different extraction cartridges. The method developed is suitable for environmental herbicide analysis.

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